Vaccine trial against canine visceral leishmaniasis in the Islamic Republic of Iran

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تجربة لقاح ضد الليشمانيات الحشوية الكلبية في جمهورية إيران الإسلامية

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خلاصة : في هذه الدراسة ، تم تقسيم 16 كلباً مختارة للبحث بطريقة عشوائية على أربع مجموعات . فتلقت المجموعة الأولى لقاح الليشمانيا الطفلية المعالج بالحرارة الموصدة مع لقاح بي سي جي . وتلقت المجموعة الثانية لقاح الليشمانيا الكبيرة المعالج بالحرارة الموصدة مع لقاح ب س ج . وتلقت المجموعة الثالثة لقاح بي سي جي وحده ، بينما تلقت المجموعة الرابعة محلولاً ملحياً عيارياً . ولقد حقنت اللقاحات في أدمة جلد الكلاب ثلاث مرات ، يفصل كلا منها عن الأخرى ثلاثون يوماً . ثم اختبرت الكلاب كل شهرين باختبار الليشمانين الجلدي واختبار إليزا المضاد لليشمانيا لاكتشاف الأضداد الجيمية IgG والميمية IgM في عينات من الدم . وبعد تسعين يوماً من إعطاء الجرعة الثالثة ، أعطيت لكل كلب جرعة تحد اختباري داخل الصفاق قدرها 2.5×10^6 من المُشيَّقات من الليشمانيا الطفلية . وبعد المتابعة لمدة ستة شهور أجريت الصفة التشريحية للكلاب (تشريح الجثة) جميعها بحثاً عن الطفيليات . ولقد وجدت جميع الكلاب التي لم تتلق اللقاحات مصابة بالليشمانيا الطفلية ، بينما لم يكن هناك إلا كلب واحد مصاب بالعدوى بين مجموعة الكلاب الملقحة .

ABSTRACT Sixteen dogs were randomly divided into four groups. Group 1 received autoclaved *Leishmania infantum* vaccine with BCG. Group 2 received autoclaved *L. major* vaccine with BCG. Group 3 received BCG alone and Group 4 received normal saline. Dogs received the vaccines intradermally three times each at 30-day intervals. All dogs were tested at 2-month intervals with the leishmanin skin test and anti-*Leishmania* ELISA. Ninety days after the third dose, each dog received an intraperitoneal challenge of 2.5×10^6 infective promastigotes of *L. infantum*. Necropsy was performed on all dogs to investigate for parasites. All of the dogs in the unvaccinated groups were infected with *L. infantum* but of the dogs in the vaccinated groups, only one dog was infected.

Essai relatif à un vaccin contre la leishmaniose viscérale canine en République islamique d'Iran
RESUME Seize chiens ont été répartis aléatoirement en quatre groupes. Les chiens du groupe 1 ont reçu un vaccin autoclavé préparé à partir de promastigotes de *Leishmania infantum* ainsi que le BCG. Aux chiens du groupe 2, on a administré un vaccin autoclavé préparé à partir de promastigotes de *L. major* ainsi que le BCG. Les chiens du groupe 3 n'ont reçu que le BCG et les chiens du groupe 4 ont reçu une solution saline normale. Les vaccins ont été administrés aux chiens par voie intradermique à trois reprises à 30 jours d'intervalle. Des tests ont été effectués sur tous les chiens à des intervalles de 2 mois en utilisant l'épreuve cutanée à la leishmanine et la méthode ELISA. Quatre-vingt-dix jours après la troisième dose, chaque chien a été inoculé par voie intrapéritonéale avec la dose d'épreuve de $2{,}5 \times 10^6$ promastigotes infectieux de *L. infantum*. Une nécroscopie a été réalisée chez tous les chiens à la recherche de parasites. Tous les chiens des groupes n'ayant pas été vaccinés contre la leishmaniose étaient infectés par *L. infantum* mais parmi les chiens des groupes ayant été vaccinés, un seul chien était infecté.

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Introduction

Canine visceral leishmaniasis (CVL) is caused by *Leishmania infantum* and is endemic throughout the northwestern and southern parts of the Islamic Republic of Iran [*1*]. Dogs appear to be the chief source of infection for human visceral leishmaniasis. Unfortunately, too many seropositive dogs exist in the endemic areas and antivector measures have been largely unsuccessful [*2*]. Treatment of infected dogs is not recommended; attempts to treat infected dogs with antimonials have given poor results. Recrudescence following treatment is common, especially in animals with symptomatic infections. Furthermore, chemotherapy for infected dogs is impractical in developing countries because of the high cost of treatment [*3,4*].

In view of the growing public health importance of zoonotic visceral leishmaniasis (ZVL) and the difficulty in controlling it, a new control strategy seems necessary. Prevention of the disease in dogs appears to be the most effective approach for interrupting the domestic cycle of ZVL. One strategy would be to develop a vaccine that protects dogs from developing parasitaemia of cutaneous infections and from becoming reservoir hosts for the parasite. Recent studies [*5–8*] of dogs experimentally infected and naturally infected with *L. chagasi* or *L. infantum* indicate that many animals survive infection with the parasite and develop a cellular immune response that probably results in resistance. These findings suggest that a canine vaccine against visceral leishmaniasis is quite feasible.

Materials and methods

The objective was to evaluate the efficacy of autoclaved *L. infantum* (ALi) and autoclaved *L. major* (ALm) vaccines in laboratory dogs with experimental challenge of promastigotes of *L. infantum*.

In this study, 16 eligible dogs were randomly divided into four groups:

- Group 1 received ALi (1 mg protein/dose) + BCG (400 µg/dose).
- Group 2 received ALm (1 mg protein/dose) + BCG (400 µg/dose).
- Group 3 received BCG (400 µg/dose).
- Group 4 received normal saline (NS).

Dosages were based on dosages used in a vaccine trial against zoonotic cutaneous leishmaniasis on human volunteers in Iran [*9,10*]. ALm was prepared from the promastigotes of *L. major* (MRHO/IR/76/ER, vaccine) [*11,12*]. Promastigotes were grown in RPMI (Gibco, Grand Island, NY, USA) with 15% fetal calf serum (Sigma, St. Louis, MO, USA) at 25 °C. Parasites were harvested at stationary phase (assessed by daily enumeration) on days 16 through 20 by centrifugation at 3200 rpm for 30 minutes. Promastigotes were washed five times with pyrogen-free phosphate-buffered saline (PBS), pH 7.0–7.2 and stored at −70 °C. The sample was divided into small vials, autoclaved for 15 minutes at 121 °C and kept at 4 °C. Lowry's method was used for protein measurement [*13*].

ALi vaccine was composed of autoclaved promastigotes of *L. infantum* (strain MCAN/IR/94/LON 49) prepared in the protozoology unit in the School of Public Health at Teheran University of Medical Sciences according to the same procedure as that used for *L. major*. Dogs received the vaccines intradermally three times each at 30-day intervals. All dogs were tested at 2-month intervals with the leishmanin skin test and anti-*Leishmania* ELISA for the detection of IgG and IgM antibodies in blood collections.

Ninety (90) days after the third dose, each dog received an intraperitoneal chal-

lenge of 2.5×10^6 infective promastigotes of *L. infantum* (strain MCAN/IR/94/LON 49). About 6 months after administering the promastigote challenge, necropsy was performed on all dogs to investigate for the parasites.

Before killing the infected dogs, two blood samples from each dog were collected in heparinized capillary tubes. These samples were tested by enzyme-linked immunosorbent assay (ELISA). Then necropsy was performed. The spleen, liver and popliteal lymph nodes of all dogs were cultured in a Novy-MacNeal-Nicolle (NMN) + liver infusion tryptose (LIT) medium and checked twice a week for six weeks.

Impression smears were prepared from the internal organs, including the spleen and the liver. The smears were stained with standard Giemsa stain and examined microscopically for the amastigote form of *Leishmania*.

Results

Table 1 shows the results of the leishmanin skin test before vaccination and after the first and third vaccinations. Table 2 shows the results of parasitological investigation following necropsy. The immunization courses did not provoke any apparent side-effects.

Discussion

The first vaccine trial against CVL was carried out by Monjour et al. [*14*], who used a synthetic antigen of *L. infantum* in 393 seronegative dogs in the south of France. The dogs were followed for two years and no differences in the rate of infection between vaccinated and unvaccinated groups were seen. Other trials using merthiolated ultrasound disrupted promastigotes of *L. braziliensis* vaccine + BCG were carried out on dogs by Genaro et al [*15*].

To evaluate the efficacy of autoclaved *Leishmania* vaccines against CVL, we conducted our experiments in two phases. The first phase, presented here, tested the vaccine in laboratory dogs with experimental challenge of promastigotes of *L. infantum*. The second phase tested the vaccine in the field where dogs receive natural challenges. The results of the latter phase of this study will be finalized in the next year.

In groups vaccinated with ALi or ALm, significant responses to the leishmanin skin tests were seen. In general, the addition of BCG to ALi or ALm enhanced the induction

Table 1 **Results of leishmanin tests before and 2 months after the first and third vaccinations**

Group	Injection	No. of dogs	Results of leishmanin skin test		
			Before vaccination (mm)	2 months after first vaccination (mm)	2 months after third vaccination (mm)
1	ALi + BCG	4	0.50	6.0	6.70
2	ALm + BCG	4	0.20	6.70	7.10
3	BCG	4	0.25	3.20	3.00
4	Normal saline	4	0.30	2.10	1.80
Total/average		16	0.31		

Table 2 **Efficacy of autoclaved *Leishmania* vaccines in dogs**

Group	Dogs	Serological results (ELISA)		Leishmanin skin test 160 days after vaccine	Parasitological results after challenge	
		After vaccine	After challenge		Smear	Culture
1	1	–	–	–	–	–
(ALi + BCG)	2	–	–	+	–	–
	3	–	–	+	–	–
	4	–	–	–	–	–
2	1	–	+	–	+	+
(ALm + BCG)	2	–	–	+	–	–
	3	–	–	+	–	–
	4	–	–	+	–	–
3	1	–	+	–	+	+
(BCG)	2	–	+	–	+	+
	3	–	+	–	+	+
	4	–	+	–	+	+
4	1	–	+	–	+	+
(normal saline)	2	–	+	–	+	+
	3	–	+	–	+	+
	4	–	+	–	+	+

– indicates negative result, + indicates positive result

of delayed hypersensitivity to leishmanin skin test conversions. The third injection of ALi and ALm resulted in stronger skin test conversions from negative to positive than the first and second vaccination. In the vaccinated groups (1 and 2) one out of eight animals showed clear *L. infantum* infection, whereas in the unvaccinated groups (3 and 4) all eight dogs developed infection. Antibodies were detected after infection by *Leishmania*. This study shows that autoclaved *L. infantum* and autoclaved *L. major* vaccine can protect dogs against active promastigotes in experimental conditions.

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